Intrauterine bacterial inoculation and level of dietary methionine alter amino acid metabolism in nulliparous yearling ewes

T. M. Thelen, C. A. Löest, J. B. Taylor, S. Wang and G. S. Lewis

J Anim Sci 2007.85:3371-3382.
doi: 10.2527/jas.2006-851 originally published online Sep 4, 2007;

Intrauterine bacterial inoculation and level of dietary methionine alter amino acid metabolism in nulliparous yearling ewes[1]

T. M. Thelen,* C. A. Löest,* J. B. Taylor,†[2] S. Wang,† and G. S. Lewis†

*Department of Animal and Range Science, New Mexico State University, Las Cruces 88003;
†USDA, ARS, US Sheep Experiment Station, Dubois, ID 83423

ABSTRACT: Using an intrauterine bacterial inoculation method, our objective was to determine the effects of acute sepsis and level of dietary metabolizable Met (MM) on splanchnic metabolism of AA in ewes. Twenty-four nulliparous yearling Rambouillet-cross ewes (initial BW = 65.1 ± 0.6 kg), surgically fitted with chronic-indwelling catheters in hepatic and portal veins, a mesenteric vein and artery, and the uterine lumen, were assigned to a 2 × 2 factorial arrangement of treatments. Factors were intrauterine bacterial inoculation (noninoculated vs. inoculated) and level of MM [low (2.28 g/d) vs. high (3.91 g/d)]. Beginning 12 h before sampling, inoculated and noninoculated ewes received 10-mL intrauterine infusions of *Escherichia coli* (9.69×10^{11} cfu) + *Arcanobacterium pyogenes* (2.76×10^{12} cfu) and of sterile saline, respectively. Uterine infection was induced in ewes that received intrauterine bacterial inoculations, but not in ewes infused with sterile saline. Bacterial inoculation resulted in increased hepatic release and plasma concentrations of aromatic AA used for acute-phase protein synthesis, increased hepatic removal and decreased plasma concentrations of AA used for glutathione synthesis, and decreased plasma concentrations of some gluconeogenic and acetogenic AA used for glucose recycling and anaerobic energy production, respectively ($P < 0.05$). In ewes fed high-MM diets, compared with low-MM diets, a consistent net hepatic uptake of Phe occurred throughout the sampling period, more Asp was released from the portal-drained viscera, and hepatic vein glucose concentrations were greater ($P < 0.05$). We conclude that Met seemed to be limiting in low-MM ewes, and as such, would continue to be limiting during sepsis. However, additional MM, in excess of the dietary requirement, would not necessarily result in a benefit to ewes experiencing acute sepsis.

Key words: methionine, amino acid, sepsis, sheep, uterine infection

J. Anim. Sci. 2007. 85:3371–3382
doi:10.2527/jas.2006-851

INTRODUCTION

Disease alters AA metabolism (for review, see Wannemacher, 1977). Site-specific use of AA may shift from one tissue to another during a host's defense against sepsis. Anabolism of skeletal-muscle protein shifts to catabolism and makes AA available for acute-phase protein synthesis, cellular proliferation, and energy. However, AA composition of skeletal muscle may not complement the AA requirement for the host's immune system to mitigate sepsis (Reeds and Jahoor, 2001). Thus, gram for gram, more muscle protein must be mobilized to support synthesis of acute phase proteins (Reeds et al., 1994). When considering AA metabolism in diseased hosts, Reeds and Jahoor (2001) astutely surmised, "amino acids..., which are not traditionally regarded as essential, become, at least potentially, limiting".

Endotoxin infused into the artery of sheep decreased venous plasma concentrations of Met and other AA (Hofford et al., 1996). When considering that Met is limiting in growing ruminants (Storm and Ørskov, 1984; Greenwood and Titgemeyer, 2000), acute sepsis may further antagonize the availability of Met. In rats given *Escherichia coli* injections (i.v.), Cys utilization for protein synthesis doubled (Malmezat et al., 2000a), with a substantial portion of Cys derived from Met transsulfuration (Malmezat et al., 2000b). Additionally, the key role of S-adenosylmethionine in cytokine production and acute-phase protein synthesis (Arias-Díaz et al., 1996; Song et al., 2002, 2005) suggests that poten-

[1]Authors wish to thank Marta Remmenga (New Mexico State University) for statistical guidance, Liang Chen (New Mexico State University) for assisting with AA analyses, and Bret W. Hess (University of Wyoming) for conducting rumen-degradable and undegradable N analyses.

[2]Corresponding author: btaylor@pw.ars.usda.gov

Received December 30, 2006.
Accepted August 6, 2007.

tial interactions between Met and acute sepsis may affect splanchnic metabolism of AA in sheep.

Therefore, using an intrauterine bacterial inoculation method (Lewis and Wulster-Radcliffe, 2006), our objective was to determine the effects of acute sepsis and level of dietary metabolizable Met (**MM**) on splanchnic metabolism of AA in nulliparous yearling ewes.

MATERIALS AND METHODS

Experimental Ewes and Design

An Institutional Animal Care and Use Committee (USDA, ARS, Dubois, ID) reviewed and approved all experimental and husbandry procedures for this study.

Twenty-four nulliparous yearling Rambouillet-cross ewes (initial BW = 65.1 ± 0.6 kg), surgically fitted with chronic-indwelling catheters in hepatic and portal veins, a mesenteric vein and artery, and uterine lumen, were housed individually (80 × 240-cm pens with slatted floors) in an enclosed facility (temperature = 22.3 ± 2.5°C) and allowed ad libitum access to water and sugar beet-pulp pellets. In a randomized complete block design, ewes were assigned (as described below) to a 2 × 2 factorial arrangement of randomized treatments. Treatment factors were intrauterine bacterial inoculation (noninoculated vs. inoculated) and level of metabolizable Met (**low MM** [2.28 g/d] vs. **high MM** [3.91 g/d]). A timeline of the experimental events is presented in Figure 1.

Surgeries

For surgical placement of catheters, ewes were anesthetized (2,747 ± 105 mg of sodium pentobarbital administered i.v. throughout surgery [198 ± 6 min]) and placed in dorsal recumbency. The uterus was elevated through a midventral incision (~5 cm), and a catheter (130 cm in length × 1.02 mm i.d. × 1.78 mm o.d. sterile polyvinyl tubing; Tygon MicroBore Tubing, Formulation S-54-HL, VWR International, Bristol, CT) was inserted (~6 cm) into the uterine lumen via a puncture wound and anchored to the uterine body. The incision was closed, and ewes were rotated to a left lateral recumbency. A paracostal incision was made approximately 3 to 5 cm caudal to the last rib. Catheters were placed 10 to 15 cm into a hepatic vein (catheter = 130 cm in length × 1.27 mm i.d. × 2.29 mm o.d. polyvinyl tubing) of the left lobe; 3 to 4 cm into the portal vein (catheter = 130 cm in length × 1.27 mm i.d. × 2.29 mm o.d. polyvinyl tubing), with a final placement that was approximately 2 cm inside the body of the liver; and 13 and 5 cm into a mesenteric artery and vein (catheters = 150 cm long × 1.27 mm i.d. × 2.29 mm o.d. polyvinyl tubing), respectively, in the second and sixth accessible caudal vascular loops of the small intestinal vasculature. All catheters were anchored at the insertion sites and exteriorized, together, near the paracostal incision and subcutaneously along the last rib through a 1-cm incision above and near the fist lumbar vertebra. Vascular catheter void volumes were filled with heparinized saline (100 U/mL), and 2 knots were tied at the exterior ends of each catheter. Ewes received 10 mL of Penicillin G Procaine i.p. (300,000 U/mL; Agri-Cillin, AgriLabs, St. Joseph, MO) before closure, and 10 mL s.c. the following morning. Surgeries were conducted over a 3-wk period (8 ewes/wk; surgery week = blocking factor). To encourage intake soon after surgery, chopped (1 cm length) alfalfa hay was fed with sugar beet-pulp pellets for 2 d (Figure 1; Table 1).

Treatment Delivery

Beginning 3 d after the surgeries, all ewes were fed a sugar beet-pulp pellet diet (Table 1) in 2 equal portions daily (0700 and 1900) at 1.3 kg of DM/d (Figure 1) throughout the experiment. Ewes were assigned to MM treatments and received an estimated 2.28 and 3.91 g of MM/d for low-MM and high-MM treatments, respectively (Table 1). Ewes assigned to high-MM treatment were provided supplemental MM from Mepron M85 (Degussa Corporation, Kennesaw, GA), a rumen-protected feed product. High-MM ewes were bolused twice daily, at feeding, with preweighed gelatin capsules (9.9 × 26.1 mm; Torpac Inc., Fairfield, NJ) containing 2.4 g of Mepron M85, which was estimated to provide an additional 1.63 g of MM/d (Table 1).

Approximately 12 d after surgeries (Figure 1), ewes received 10-mL intrauterine infusions of either 9.69×10^{11} cfu of *Escherichia coli* + 2.76×10^{12} cfu of *Arcanobacterium pyogenes* or of sterile saline, according to the inoculation treatment assignment. The bacteria were originally obtained from a dairy cow, purified, and stored (Seals et al., 2002). To ensure susceptibility to sepsis, ewes were injected (i.m.) with 5 mg of progesterone (2 mg/mL of canola oil, i.m.) twice daily at feeding, beginning 5 d before intrauterine infusions and continuing throughout the experiment (Lewis and Wulster-Radcliffe, 2006).

Sampling

Sampling began 12 h after intrauterine infusions (refer to Figure 1). A primed (15 mL) and constant infusion (0.57 mL/min; KDS 220 MultiSyringe Pump, KD Scientific, Holliston, MA) of paraaminohippurate (**PAH**; 3% wt/vol, pH 7.4) was administered, beginning 1 h before sampling, into the mesenteric vein for estimation of venous and arterial plasma flows. Before feeding (h 0) and every 2 h throughout the 12-h sampling period, simultaneous artery, portal vein, and hepatic vein blood samples were collected into 6-mL syringes. Blood was transferred (6 mL) immediately to tubes containing 12.15 mg of EDTA (BD Vacutainer, Becton, Dickinson and Company, Franklin Lakes, NJ). Blood samples were centrifuged (1,500 × *g*, 30 min, 4°C), and plasma was removed and stored (–20°C). At h 0 and 6, simulta-

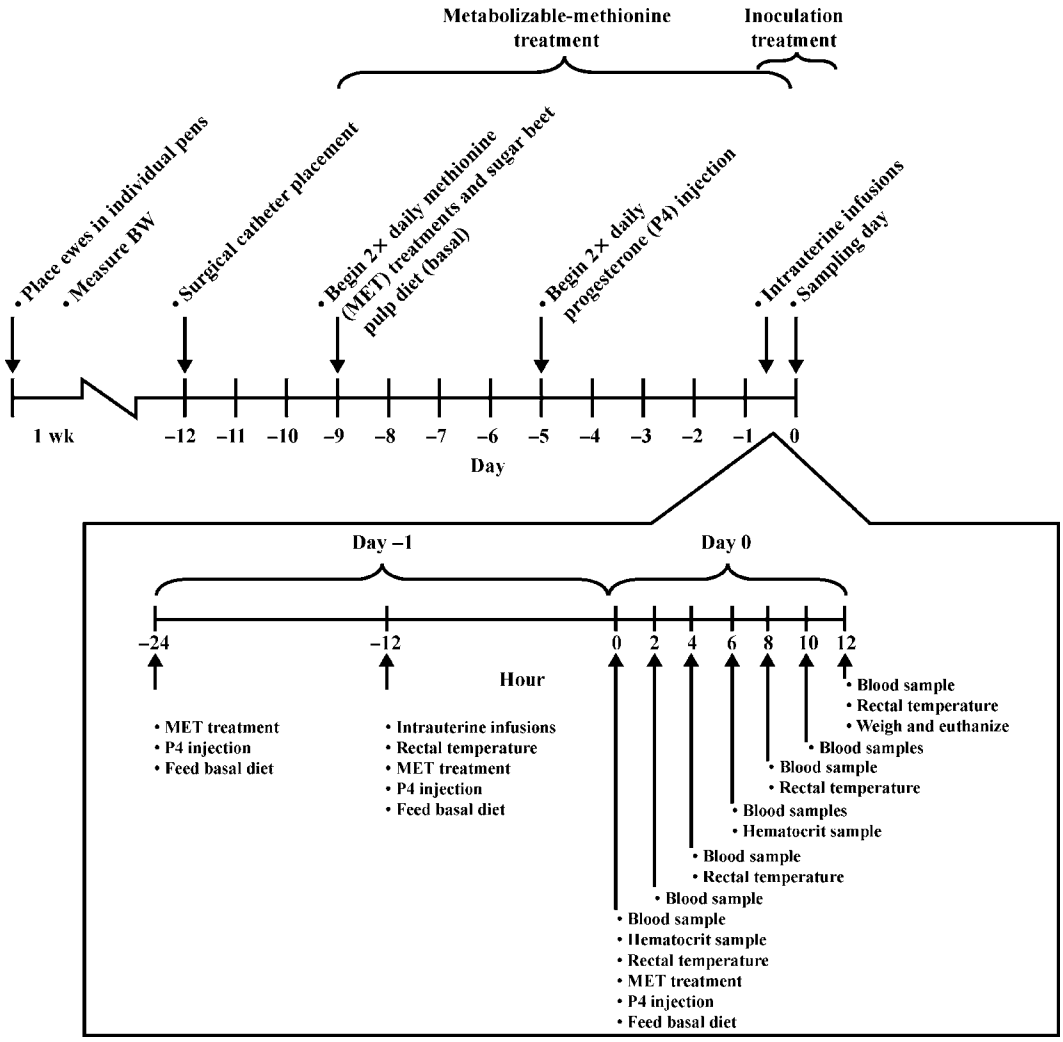

Figure 1. Experiment timeline, which includes surgeries, recovery, intrauterine infusion treatment, commencement of metabolizable Met treatment, and sampling schedule.

neous artery and hepatic vein blood samples were collected in 1-mL syringes tipped with ~40 μL of heparinized saline (1,000 U/mL) and immediately analyzed for hemoglobin (Type B Roche Opti CCA cassettes, AVL OPTI Critical Care Analyzer; Osmetech Inc., Roswell, GA; inter- and intraassay CV < 0.01%). Neither frequency nor volume of sampling altered (P = 0.08 to 0.67) artery or hepatic-vein blood hematocrit (based on hemoglobin; data not shown). Rectal temperatures were measured immediately before intrauterine infusions (h −12) and then again at feeding (h 0) and every 4 h throughout the duration of the 12-h sampling period.

Sample Analyses

Plasma concentrations of PAH, AA (His, Ile, Leu, Lys, Met, Phe, Thr, Trp, Val, Ala, Asn, Asp, Cys, Glu, Gln, Gly, Orn, Pro, Ser, and Tyr), glucose, and lactate were analyzed for all vessel samples from each sampling time. Plasma PAH (inter- and intraassay CV < 5.8%) was measured according to the method of Huntington (1982) and with modifications of Taylor (2000). Plasma glucose (inter- and intraassay CV < 4.0%; kit #G7517, Pointe Scientific Inc., Lincoln Park, MI, and based on the method of Trinder, 1969), and lactate (inter- and intraassay CV < 3.8%; kit #L7596, Pointe Scientific Inc., and based on the method of Noll, 1974) were measured using prepared kits. Plasma AA (CV < 8%) were measured according to the method of Chen et al. (2002) with the following modifications. Briefly, the amount of methanol, methanol:0.1 M HCl solution (vol/vol), and 0.1 M HCl used to prepare the SCX column was increased to 2.5 mL. The amount of sample and standard was 250 μL, with 5 μL of L-norvaline as the internal standard, and 900 μL of 1 M ammonium hydroxide was used to elute the AA. Due to difficulties (CV > 10%) in initial analysis attempts and depleted sample, plasma subsamples within ewe and vessel were pooled across sampling times for final ammonia and urea analyses. Urea (inter- and intraassay CV < 1.8%; kit #DIUR, BioAssay Systems, Hayward, CA, and based on the method described by Jung et al., 1975) and ammonia (inter- and intraassay CV < 7.1%; kit AA0100, Sigma-Aldrich Inc., Saint Louis, MO, and based on the method

Table 1. Feedstuffs, diet composition (DM basis), and daily metabolizable Met (MM) intake

Item	Treatment	
	Low MM	High MM
Feedstuffs, %		
Sugar beet-pulp pellet	99.3	99.3
Trace mineral salt[1]	0.62	0.62
Vitamin E mix[2]	0.06	0.06
Diet composition		
ADF,[3] %	23.2	23.2
TDN,[4] %	70.8	70.8
CP, %	10.7	10.7
UIP,[5] % of CP	54.4	54.4
Total MM, g/d	2.28	3.91
MM_{UIP}[6]	0.39	0.39
MM_{MCP}[6]	1.89	1.89
MM supplemented[7]	0	1.63

[1]95.0% NaCl, 0.55% Ca, 0.0007% Cu, 0.002% I, 0.07% Fe, 0.09% Mg, 0.0007% Mn, 0.05% P, 0.12% K, and 0.13% S; DM basis (Redmond NTM salt, Redmond, UT).
[2]6,287 IU of vitamin E/kg (E-5000, Vita-Flex Nutrition, Council Bluffs, IA).
[3]AOAC (1990) Method No. 973.18.
[4]TDN = 88.9 – (ADF, % × 0.779).
[5]Undegradable intake protein (Mortenson et al., 2005).
[6]MM_{UIP} = Met provided by dehydrated sugar beet pulp (0.65%) × UIP, % of CP × 0.8 digestibility (NRC, 1996). MM_{MCP} = microbial CP (13% of dietary TDN; NRC, 1996) × 0.64 g of digestible AA/g microbial CP × 0.0247 g of Met/g of AA (Storm and Ørskov, 1983).
[7]Supplemental MM provided as Mepron M85 (Degussa Corporation, Kennesaw, GA), which is a high-Met rumen-protected product.

of Neeley and Phillipson, 1988) concentrations were measured using prepared kits.

Postmortem Sampling and Examination

All animals were euthanized (captive bolt and exsanguination) after the last sampling (h 12; Figure 1), reproductive tracts were collected, and catheter placement was examined. To determine presence of infection, uterine contents were flushed with 15 mL of sterile saline (Lewis and Wulster-Radcliffe, 2006). Flushing turbidity (as determined by measuring the optical density at 560 nm) and sediment (centrifugation, 1,500 × *g* for 10 min) were measured. The uterus was opened, the luminal surface was scraped with a sterile-cotton swab, and swab contents were transferred to a culture plate (Columbia SB Agar, Becton, Dickinson, and CO., Sparks, MD) and incubated (12 h at 37°C). The plates were examined for bacterial growth (absent or present). Clear uterine flushings, small amounts of sediment (<5% by volume), which contained bacteria and leukocytes, no signs of endometrial inflammation, and the inability to culture dense colonies of bacteria from the flushings were signs that a uterus was not infected. Cloudy uterine flushings, large amounts of sediment (>5%, but usually >20%, by volume), an inflamed endometrium, and the ability to culture dense colonies of bacteria from the flushings were signs of infection. This method for determining whether a sheep uterus contained an infection and then classifying the severity of the infection is consistent with a method used for cervical mucus from cattle (Sheldon et al., 2006).

Calculations and Statistics

Due to improper catheter placement and catheter patency complications, some sample analysis values were excluded from the data set or missing. For example, hepatic vein catheters were placed in the vena cava of 3 ewes, and artery, hepatic vein, and portal vein catheter patency were lost periodically in some ewes during sampling. The greatest incidence of patency complications occurred at h 6, resulting in removal of all data corresponding to h 6. These incidences resulted in decreased replication, especially when plasma flow rates and metabolite/AA fluxes were calculated. Therefore, for some measures, our ability to detect treatment effects or treatment interactions was limited. Experimental units for data corresponding to each vessel concentration, plasma flow, flux, and hepatic extraction ratio are presented in Table 2.

Portal-vein, hepatic-vein, and artery plasma-flow rates (based on the Fick principle), fluxes, and hepatic-extraction-ratios were calculated as follows:

$$\text{portal-vein plasma flow (}\mathbf{PPF}\text{)} = IR / (Cp - Ca),$$

$$\text{hepatic-vein plasma flow (}\mathbf{HPF}\text{)} = IR / (Ch - Ca), \text{ and}$$

$$\text{hepatic artery plasma flow (}\mathbf{APF}\text{)} = HPF - PPF,$$

where IR is the PAH infusion rate (g/h) into the mesenteric vein and Cp, Ch, and Ca are PAH concentrations (g/L) in portal-vein, hepatic-vein, and hepatic artery plasma, respectively; in addition,

$$\text{portal-drained viscera (}\mathbf{PDV}\text{) flux} = PPF \times (Cp - Ca),$$

$$\text{hepatic flux} = [PPF \times (Ch - Cp)] + [APF \times (Ch - Ca)],$$

$$\text{total splanchnic-tissue (}\mathbf{TST}\text{) flux} = \text{PDV flux} + \text{hepatic flux, and}$$

$$\text{hepatic-extraction ratio (}\mathbf{HER}\text{)} = \text{hepatic flux} / [(PPF \times Cp) + (APF \times Ca)],$$

where Ca, Cp, and Ch are the AA and metabolite concentrations (m*M*) in the artery, portal vein, and hepatic vein plasma samples, respectively.

Dry matter intakes (after uterine infusion), BW (final), uterine-sepsis indicators (uterine flush turbidity and solids), and plasma urea and ammonia concentrations, fluxes, and HER were analyzed using mixed models [MIXED procedure, SAS Inst. Inc., Cary, NC; denominator degrees of freedom (**ddf**) = Satterthwaite]. The model included the treatment factors and all interactions as fixed effects, and surgery week as a block. Plasma-flow rates, and plasma AA, glucose, and lactate concentrations, fluxes, and HER, and rectal tempera-

Table 2. Number of experimental units for data analyses corresponding to each vessel concentration, plasma flow, flux, and hepaticextraction ratio

	Metabolizable Met treatment[1]			
	Low		High	
	Inoculation treatment[2]			
Item	No	Yes	No	Yes
Vessel				
Artery	4	4	5	5
Hepatic vein	4	2	5	5
Portal vein	5	4	6	6
Plasma flow				
Artery	4	3	4	4
Hepatic vein	4	3	4	4
Portal vein	4	4	5	4
Flux[3]				
PDV[4]	4	4	5	4
Hepatic	3	2	4	4
TST[4]	3	2	4	4
HER[5]	3	2	4	4

[1]Low = 2.28 g metabolizable Met/d; High = 3.91 g metabolizable Met/d.
[2]No = intrauterine infusion of sterile saline; Yes = intrauterine bacterial inoculation (9.69×10^{11} cfu of *Escherichia coli* and 2.76×10^{12} cfu of *Arcanobacterium pyogenes*).
[3]For low-metabolizable Met × no inoculation ewes, experimental units for hepatic and TST ammonia and urea fluxes were 4.
[4]PDV = portal-drained viscera; TST = total-splanchnic tissue.
[5]HER = hepatic extraction ratio.

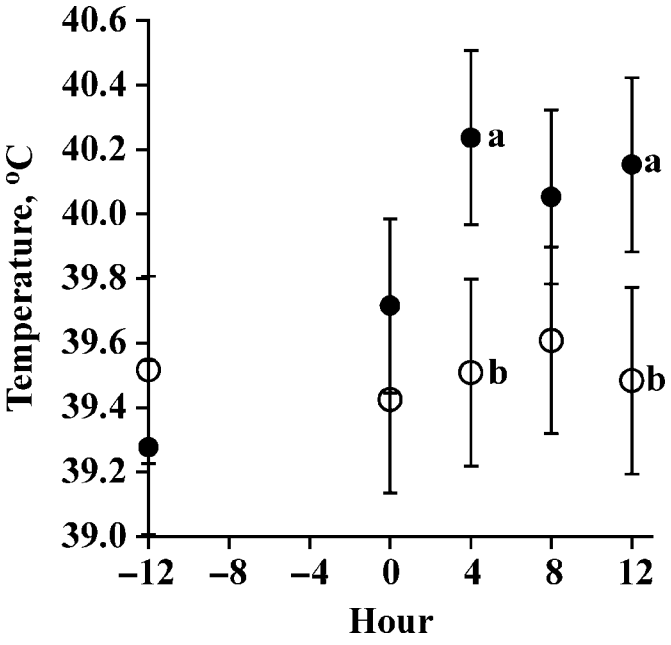

Figure 2. Rectal temperatures [least squares means ± SEM (bars)] of nulliparous yearling Rambouillet crossbred ewes that received an intrauterine sterile saline infusion (○) or bacterial inoculation (●; 9.69×10^{11} cfu of *Escherichia coli* and 2.76×10^{12} cfu of *Arcanobacterium pyogenes*). Sampling time × inoculation interaction was significant ($P < 0.01$). [a,b]Means with unlike superscripts within each sampling time are different ($P < 0.01$).

ture data were treated as repeated measures and also were analyzed using mixed models with the MIXED procedure of SAS (ddf = Satterthwaite; covariance structure = autoregressive order 1 for plasma-flow rates and spatial power law for all other response variables). The model included treatment factors, sampling time, and all interactions as fixed effects, and surgery week as a block. Effects were considered significant when the probability of a greater *F*-value was < 0.05. Mean separations were performed using planned pairwise *t*-tests when the *F*-tests were significant.

RESULTS

Sepsis Verification and Ewe Performance

Neither the inoculation × MM interaction ($P = 0.40$ to 0.78) nor MM main effect for uterine-sepsis indicators were significant ($P = 0.49$ to 0.91). However, uterine sepsis was induced in ewes that received intrauterine-bacterial inoculations when compared with those that received intrauterine-saline infusions. Specifically, inoculated ewes had greater rectal temperatures ($P < 0.05$; Figure 2) following inoculation, and subsequent uterine flushings were greater in volume ($P < 0.01$), more turbid ($P < 0.01$), and resulted in larger pellets following centrifugation ($P < 0.01$; Table 3) compared with noninoculated. Uteri from inoculated ewes appeared inflamed and had apparent pustule-like sites throughout the uterine lumen. Bacteria were present only in cultures from uteri inoculated with *E. coli* and *A. pyogenes*. Neither the treatment main effects nor the inoculation × MM interaction was significant for ewe BW and DMI on the day of sampling ($P = 0.59$ to 0.93). The grand mean BW and DMI (for the 12-h sampling period) was 60.4 ± 0.6 and 0.60 ± 0.03 kg, respectively.

Plasma Flow Rates

Although the inoculation × MM interaction was significant ($P = 0.03$) for hepatic vein plasma-flow rates, no differences ($P = 0.07$ to 0.92) among factor-combination means were detected. The detected interaction seemed to be because of a numerically lower hepatic vein plasma flow rate (124 ± 11 L/h) for the low-MM, noninoculated ewes than for ewes from other factor-combination treatments (data not shown). Neither the sampling time × treatment interactions nor treatment main effects were significant for hepatic artery and portal-vein plasma flow rates ($P = 0.06$ to 0.99). Grand mean plasma-flow rates were: portal vein = 106.6 ± 2.9 L/h, hepatic vein = 137.2 ± 3.3 L/h, hepatic artery = 32.8 ± 1.7 L/h.

AA and Metabolite Concentrations

The inoculation × MM interactions were significant for artery plasma Trp and Gln concentrations ($P < 0.05$;

Table 3. Indicators (least squares means) of sepsis in nulliparous yearling Rambouillet crossbred ewes that received either an intrauterine sterile-saline infusion or intrauterine bacterial inoculation

	Inoculation treatment[1]			
Item	No	Yes	SEM	*P*-value[2]
Number of uteri	12	11	—	—
Flush volume,[3] mL	10.4	13.7	0.80	<0.01
Turbidity,[4] OD	0.19	2.22	0.12	<0.01
Sediment depth,[5] cm	0.2	2.1	0.4	<0.01
Bacterial presence in uterine cultures[6]	0/12	11/11	—	—

[1]No = intrauterine infusion of sterile saline; Yes = intrauterine bacterial inoculation (9.69×10^{11} cfu of *Escherichia coli* and 2.76×10^{12} cfu of *Arcanobacterium pyogenes*). Inoculation treatments occurred 12 h before commencement of blood sampling.
[2]*F*-test probability for effect of inoculation.
[3]Content volumes collected from the uterus after an intrauterine flush (postmortem) with sterile saline (15 mL).
[4]OD = optical density (560 nm) of the uterine flushing.
[5]Sediment depth (cm) of flushings was measured after centrifugation ($1{,}500 \times g$, 10 min).
[6]Visual presence of bacterial growth expressed as counts of positive cultures per total counted cultures.

Table 4). Noninoculated × high-MM ewes had less artery plasma Trp than inoculated × high-MM ewes ($P = 0.04$). Although the interaction was significant ($P = 0.03$), differences ($P = 0.08$ to 0.90) between factor-combination treatments were not detected for artery plasma Gln. The interaction seemed to be due to numerically lower artery plasma Gln in the inoculated × low-MM and noninoculated × high-MM ewes compared with noninoculated × low-MM and inoculated × high-MM ewes. Inoculated ewes had less artery plasma Leu, Met, Thr, Ala, Asn, Gly, Pro, and Ser, but greater Phe than noninoculated ewes ($P < 0.05$; Table 5). Neither the sampling time × treatment interactions nor treatment main effects were significant for artery plasma His, Ile, Lys, Val, Asp, Cys, Glu, Orn, Tyr, ammonia, glucose, lactate, or urea ($P = 0.07$ to 0.99); grand means are reported in Table 6.

The sampling time × inoculation interaction was significant for hepatic-vein plasma Lys, Tyr, His, Trp, Gln, and Orn ($P < 0.05$). At h 8, inoculated ewes, compared with noninoculated, had less ($P < 0.04$) hepatic-vein plasma Lys (0.165 ± 0.085 vs. 0.291 ± 0.082 m*M*) and Tyr (0.045 ± 0.015 vs. 0.071 ± 0.014 m*M*). Although the interaction between sampling time and inoculation was significant ($P < 0.04$), inoculation treatment differences in hepatic-vein plasma His, Trp, Gln, and Orn were not detected within any sampling time ($P = 0.08$ to 0.96); as such, grand means are reported (Table 6). The sampling time × MM interaction was significant for hepatic-vein plasma lactate concentration ($P < 0.01$). In high-MM ewes, compared with low-MM, hepatic-vein plasma lactate was greater ($P = 0.05$) at h 2 (0.935 ± 0.080 vs. 0.742 ± 0.098 m*M*) but less ($P < 0.01$) at h 12 (0.680 ± 0.082 vs. 0.975 ± 0.099 m*M*). Inoculated ewes had less ($P < 0.04$) hepatic-vein plasma Ala, Glu, Gly, and Pro than noninoculated ewes (Table 5), whereas high-MM ewes had greater ($P = 0.03$) hepatic-vein plasma glucose than low-MM ewes (4.41 ± 0.16 vs. 3.72 ± 0.22 m*M*). Neither the sampling time × treatment interactions nor treatment main effects were significant for hepatic-vein plasma Ile, Leu, Met, Phe, Thr, Val, Asn, Asp, Cys, Ser, ammonia, and urea ($P = 0.06$ to 0.99); grand means are reported in Table 6.

The sampling time × MM interaction was significant for portal-vein plasma Gly ($P < 0.01$). However, no differences between treatments occurred at any sampling

Table 4. Mean (least squares) plasma AA concentrations and metabolite fluxes in nulliparous yearling Rambouillet-cross ewes that were fed diets low or high in metabolizable Met[1] and received an intrauterine sterile-saline infusion or intrauterine bacterial inoculation[2]

	Metabolizable Met treatment[1]					
	Low		High			
	Inoculation treatment[2]					
Item	No	Yes	No	Yes	SEM	*P*-value[3]
Artery concentration, m*M*						
Trp	0.045[ab]	0.041[ab]	0.036[a]	0.047[b]	0.004	0.05
Gln	0.287	0.212	0.217	0.269	0.040	0.03
PDV[4] flux, mmol/h						
Ammonia	21.8[a]	13.7[b]	14.7[ab]	20.6[ab]	2.8	0.01
TST[5] flux, mmol/h						
Ammonia	–0.56[a]	–3.53[b]	–2.00[ab]	–1.49[ab]	0.99	0.05

[a,b]Means with unlike superscripts within a row are different ($P < 0.04$).
[1]Low = 2.28 g of metabolizable Met/d; high = 3.91 g of metabolizable Met/d.
[2]No = intrauterine infusion of sterile saline; Yes = intrauterine bacteria inoculation (9.69×10^{11} cfu of *Escherichia coli* and 2.76×10^{12} cfu of *Arcanobacterium pyogenes*). Inoculation treatments occurred 12 h before commencement of blood sampling.
[3]*F*-test probability for the metabolizable Met × inoculation interaction.
[4]PDV = portal-drained viscera.
[5]TST = total splanchnic tissue.

Table 5. Mean (least squares) plasma AA concentrations and AA and metabolite fluxes in nulliparous yearling Rambouillet-cross ewes that received an intrauterine sterile-saline infusion or intrauterine bacterial inoculation

	Inoculation treatment[1]			
Item	No	Yes	SEM	*P*-value[2]
Artery concentration, m*M*				
Leu	0.122	0.097	0.008	0.04
Met	0.018	0.012	0.002	<0.01
Phe	0.046	0.052	0.003	0.05
Thr	0.144	0.093	0.016	0.04
Ala	0.185	0.139	0.011	<0.01
Asn	0.036	0.026	0.003	0.05
Gly	0.511	0.371	0.029	<0.01
Pro	0.095	0.065	0.005	<0.01
Ser	0.102	0.059	0.020	<0.01
Hepatic-vein concentration, m*M*				
Ala	0.168	0.114	0.017	<0.01
Glu	0.146	0.114	0.027	0.03
Gly	0.506	0.364	0.052	0.02
Pro	0.098	0.071	0.012	0.04
Portal-vein concentration, m*M*				
Met	0.022	0.016	0.003	0.03
Phe	0.053	0.060	0.004	0.05
Ala	0.215	0.163	0.012	<0.01
Gly	0.539	0.394	0.037	<0.01
Pro	0.106	0.079	0.008	0.02
Ser	0.128	0.087	0.017	0.02
PDV[3] flux, mmol/h				
Lactate	4.33	8.89	1.2	<0.01
Asp	0.331	0.051	0.17	0.04

[1]No = intrauterine infusion of sterile saline; Yes = intrauterine bacteria inoculation (9.69×10^{11} cfu of *Escherichia coli* and 2.76×10^{12} cfu of *Arcanobacterium pyogenes*). Inoculation treatments occurred 12 h before commencement of blood sampling.
[2]*F*-test probability for effect of inoculation.
[3]PDV = portal-drained viscera.

time (data not shown; $P = 0.12$ to 0.84). High-MM ewes, compared with low-MM, had greater portal-vein plasma Phe concentration (0.061 ± 0.004 vs. 0.053 ± 0.005 m*M*; $P = 0.03$). Inoculated ewes, compared with noninoculated, had less portal-vein plasma Met, Ala, Gly, Ser, and Pro, and greater Phe ($P < 0.05$; Table 5). Neither the sampling time × treatment interactions nor treatment main effects were significant for portal-vein plasma His, Ile, Leu, Lys, Thr, Trp, Val, Asn, Asp, Cys, Glu, Gln, Orn, Tyr, ammonia, glucose, lactate, and urea ($P = 0.07$ to 0.99); grand means are reported in Table 6.

Splanchnic-Tissue Fluxes and Hepatic Extraction Ratio

The sampling time × inoculation × MM interaction was significant for PDV-glucose flux (Figure 3; $P = 0.05$). The interaction was partly because of noninoculated, low-MM ewes having a different ($P = 0.02$) and positive PDV-glucose flux compared with noninoculated, high-MM ewes at h 12. This response would not be expected, because similar differences did not occur at previous sampling times ($P = 0.06$ to 0.99) and because the PDV-glucose flux of noninoculated, low-MM ewes did not return to near h 0 values. Based on PDV-glucose fluxes of noninoculated, high-MM ewes at h 0 and 12, additional MM, when uterine sepsis is not present, seems to result in a significant PDV uptake of glucose immediately before feeding. Neither a significant ($P = 0.06$ to 0.84) net uptake nor release of glucose from the PDV was detected in ewes from other factor-combination treatments. The sampling time × MM interaction was significant for PDV-Gly flux (Figure 4; $P = 0.02$). At h 10, Gly flux in low-MM ewes was negative and different ($P < 0.01$) from high-MM ewes. The inoculation × MM interaction was significant for PDV-ammonia flux ($P = 0.01$; Table 4). When MM was low, intrauterine bacterial inoculation, compared with no inoculation, resulted in less ammonia being released from the PDV ($P = 0.04$). The inoculation effect was significant for PDV fluxes of lactate and Asp ($P < 0.04$; Table 5). Intrauterine bacterial inoculation, compared with no inoculation, resulted in more lactate but less Asp being released from the PDV. Neither the sampling time × treatment interactions nor treatment main effects were significant for PDV flux of His, Ile, Leu, Lys, Met, Phe, Thr, Trp, Val, Ala, Asn, Cys, Glu, Gln, Orn, Pro, Ser, Tyr, and urea ($P = 0.07$ to 0.98); grand means are reported in Table 6.

The sampling time × inoculation interaction was significant for Gly hepatic flux ($P = 0.05$; Figure 5). At h 8, noninoculated ewes had greater Gly hepatic flux than inoculated ewes ($P < 0.01$), which indicated that intrauterine-bacterial inoculation suppressed the hepatic release of Gly ($P < 0.01$) at this sampling time. The sampling time × MM interaction was significant for Phe hepatic flux ($P = 0.04$; Figure 6); however, no treatment differences were detected within any sampling time ($P = 0.10$ to 0.87). Generally, there was a net uptake of Phe in both MM treatment groups, except at h 4 for high-MM and h 2 and 8 for low-MM ewes. The MM treatment effect was significant for hepatic Asp flux ($P = 0.03$). The Asp flux was greater in high-MM ewes, compared with low-MM (0.168 vs. –0.028 ± 0.061 mmol/h, respectively). Overall, additional MM resulted in a significant net release, as opposed to neither a net uptake nor release of Asp from the hepatic tissue. Neither the sampling time × treatment interactions nor treatment main effects were significant for hepatic fluxes of His, Ile, Leu, Lys, Met, Thr, Trp, Val, Ala, Asn, Cys, Glu, Gln, Orn, Pro, Ser, Tyr, ammonia, glucose, lactate, and urea ($P = 0.06$ to 0.99); grand means are reported in Table 6.

The sampling time × inoculation × MM interaction was significant for TST-Asp flux ($P = 0.05$). At h 0, TST-Asp flux was less in low-MM × inoculation treatment ewes than in ewes from all other factor-combination treatments (data not shown; $P < 0.01$; grand mean is reported in Table 6). The sampling time × inoculation interaction was significant for TST-Gln flux ($P = 0.05$), but no inoculation treatment differences were detected at any sampling time ($P = 0.06$ to 0.92; grand mean is reported in Table 6). The inoculation × MM interaction

Table 6. Grand means (±SEM) of AA and metabolite vessel concentrations and splanchnic fluxes in nulliparous yearling Rambouillet crossbred ewes[1]

Item	Concentration, m*M*			Flux,[2] mmol/h			
	Artery	Hepatic vein	Portal vein	PDV	Hepatic	TST	HER[2]
His[3]	0.048 ± 0.003	0.054 ± 0.003	0.061 ± 0.003	0.506 ± 0.24	–0.848 ± 0.31	–0.568 ± 0.23	0.028 ± 0.090
Ile	0.075 ± 0.003	0.092 ± 0.004	0.092 ± 0.003	1.32 ± 0.12	0.126 ± 0.26	1.45 ± 0.27	0.072 ± 0.085
Leu	[10]	0.132 ± 0.005	0.131 ± 0.004	1.79 ± 0.17	0.062 ± 0.33	1.86 ± 0.35	0.030 ± 0.047
Lys	0.142 ± 0.008	[7]	0.188 ± 0.011	2.01 ± 0.60	–0.177 ± 1.0	1.90 ± 0.79	0.097 ± 0.12
Met	[10]	0.017 ± 0.001	[10]	0.425 ± 0.06	–0.167 ± 0.061	0.212 ± 0.06	–0.070 ± 0.046
Phe	[10]	0.048 ± 0.001	[9, 10]	0.830 ± 0.09	[6]	–0.189 ± 0.11	[5, 6]
Thr	[10]	0.147 ± 0.009	0.142 ± 0.008	0.936 ± 0.53	–1.43 ± 0.59	–0.427 ± 0.87	–0.005 ± 0.082
Trp[3]	[8]	0.046 ± 0.002	0.045 ± 0.002	0.122 ± 0.10	–0.194 ± 0.15	–0.189 ± 0.12	–0.015 ± 0.027
Val	0.230 ± 0.007	0.258 ± 0.009	0.253 ± 0.007	1.28 ± 0.37	–0.314 ± 0.55	1.12 ± 0.56	0.012 ± 0.026
Ala	[10]	[10]	[10]	2.76 ± 0.27	–4.54 ± 0.40	–1.65 ± 0.42	–0.220 ± 0.021
Asn	[10]	0.038 ± 0.002	0.045 ± 0.002	1.29 ± 0.13	–0.912 ± 0.18	0.334 ± 0.16	–0.119 ± 0.077
Asp[4]	0.004 ± 0.001	0.005 ± 0.001	0.005 ± 0.001	[10]	[9]	0.134 ± 0.07	0.315 ± 0.065
Cys	0.006 ± 0.001	0.006 ± 0.001	0.007 ± 0.001	0.235 ± 0.14	–0.107 ± 0.10	0.020 ± 0.05	0.225 ± 0.19
Glu	0.103 ± 0.005	[10]	0.112 ± 0.004	0.343 ± 0.38	2.49 ± 0.61	3.24 ± 0.58	0.205 ± 0.042
Gln[3]	[8]	0.261 ± 0.012	0.286 ± 0.011	3.36 ± 1.0	–2.30 ± 1.4	0.657 ± 1.0	0.008 ± 0.073
Gly	[10]	[10]	[6, 10]	[6]	[7]	–2.05 ± 1.2	–0.070 ± 0.019
Orn[3]	0.159 ± 0.008	0.217 ± 0.015	0.190 ± 0.011	0.478 ± 0.62	2.59 ± 1.1	2.92 ± 0.93	0.218 ± 0.093
Pro	[10]	[10]	[10]	0.975 ± 0.13	–0.555 ± 0.22	0.441 ± 0.23	–0.045 ± 0.029
Ser	[10]	0.092 ± 0.005	[10]	2.05 ± 0.33	–2.45 ± 0.50	–0.603 ± 0.44	–0.138 ± 0.050
Tyr	0.053 ± 0.002	[7]	0.063 ± 0.002	0.698 ± 0.15	–0.977 ± 0.24	–0.323 ± 0.23	–0.090 ± 0.034
Ammonia	0.122 ± 0.005	0.115 ± 0.008	0.278 ± 0.011	[8]	–19.1 ± 1.6	[8]	–0.567 ± 0.026
Glucose	3.97 ± 0.07	[9]	3.89 ± 0.06	[5]	48.5 ± 7.4	45.6 ± 7.0	0.100 ± 0.013
Lactate	0.804 ± 0.023	[6]	0.906 ± 0.03	[10]	–4.38 ± 1.3	2.34 ± 1.5	–0.046 ± 0.010
Urea	4.02 ± 0.16	3.87 ± 0.18	3.70 ± 0.17	–21.2 ± 7.3	29.0 ± 21	14.0 ± 17	0.060 ± 0.036

[1]Except for denoted (footnotes 3 through 10) response variables, neither sampling time × treatment interactions nor treatment main effects were significant ($P = 0.06$ to 0.99).
[2]PDV = portal-drained viscera; TST = total-splanchnic tissues; HER = hepatic extraction ratio.
[3]The sampling time × inoculation interaction was significant ($P < 0.05$) for hepatic vein concentrations of His, Trp, Gln, and Orn and total splanchnic tissue flux of Gln; however, no treatment differences were detected ($P = 0.08$ to 0.96) within any sampling time. For reference, the grand means are reported.
[4]The sampling time × metabolizable Met × inoculation interaction for total splanchnic tissue flux of Asp was significant ($P = 0.03$), which is discussed in the text. For reference, the grand mean is reported.
[5]The sampling time × metabolizable Met × inoculation interactions were significant ($P < 0.05$). Data are reported in the text and figures.
[6]The sampling time × metabolizable Met interactions were significant ($P < 0.05$). Data are reported in the text and figures.
[7]The sampling time × inoculation interactions were significant ($P < 0.05$). Data are reported in the text and figures.
[8]The metabolizable Met × inoculation interactions were significant ($P < 0.05$). Data are reported in Table 4.
[9]The metabolizable Met effects were significant ($P < 0.05$). Data are reported in the text.
[10]The inoculation effects were significant ($P < 0.05$). Data are reported in Table 5.

was significant for TST-ammonia flux ($P = 0.05$). When MM was low, the net uptake of ammonia (negative flux) was greater in inoculated compared with noninoculated ewes ($P = 0.03$), but when MM was high, this effect did not occur (Table 4). Neither the sampling time × treatment interactions nor treatment main effects were significant for TST fluxes of His, Ile, Leu, Lys, Met, Phe, Thr, Trp, Val, Ala, Asn, Cys, Glu, Gly, Orn, Pro, Ser, Tyr, glucose, lactate, and urea ($P = 0.09$ to 0.98); grand means are reported in Table 6.

The sampling time × MM × inoculation interaction was significant for the Phe HER (Figure 7; $P = 0.03$). Phenylalanine HER was greatest and positive in inoculated, low-MM ewes at h 2 and in inoculated, high-MM ewes at h 4 ($P < 0.05$). Regardless of level of MM, there was a brief and early (after a meal) proportional contribution of Phe to the plasma from the hepatic tissue of inoculated ewes. This response is different in noninoculated ewes, in which a consistent hepatic consumption of Phe was maintained. Neither the sampling time × treatment interactions nor treatment main effects were significant for HER of ammonia, glucose, lactate, urea, or AA other than Phe ($P = 0.07$ to 1.00); the grand means are reported in Table 6.

DISCUSSION

Successful Bacterial Inoculation

An experimental goal for this study was to coordinate measurement of AA metabolism during acute stages of sepsis. Based on body temperature, an apparent inflammatory response was noticeable in inoculated ewes at 16 h after inoculation, which corresponded to the h-4 sampling time (Figures 1 and 3). The decision to use this method to induce controlled sepsis was based on recent work in which intrauterine bacterial inoculation was used to quantify immune response to sepsis imposed at the uterine level (Ramadan et al., 1997; Seals et al., 2003; Lewis and Wulster-Radcliffe, 2006). Spe-

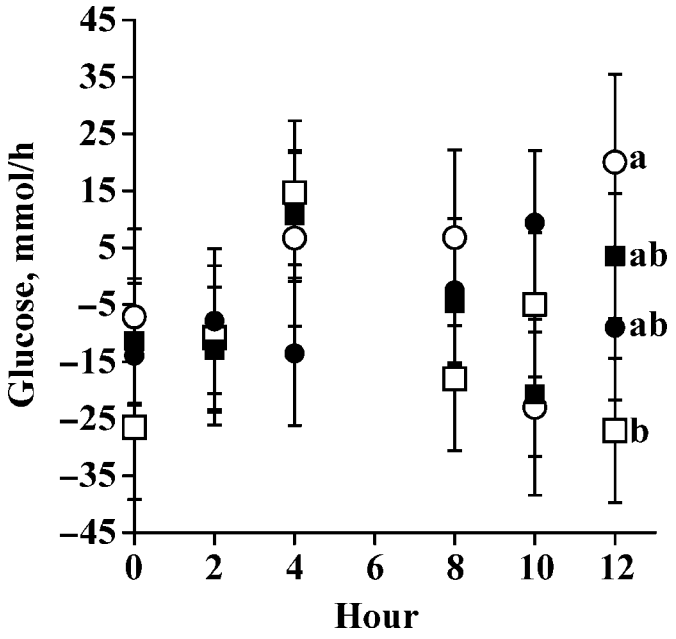

Figure 3. Portal-drained viscera flux [least squares means ± SEM (bars)] of glucose in nulliparous yearling Rambouillet crossbred ewes that were: 1) fed low dietary metabolizable Met (2.28 g/d) and received an intrauterine saline infusion (○), 2) fed high metabolizable Met (3.91 g/d) and received an intrauterine saline infusion (□), 3) fed low dietary metabolizable Met (2.28 g/d) and received an intrauterine bacterial inoculation (●; 9.69×10^{11} cfu of *Escherichia coli* and 2.76×10^{12} cfu of *Arcanobacterium pyogenes*), or 4) fed high metabolizable Met (3.91 g/d) and received an intrauterine bacterial inoculation (■; 9.69×10^{11} cfu of *E. coli* and 2.76×10^{12} cfu of *A. pyogenes*). Sampling time × metabolizable Met × inoculation interaction was significant ($P = 0.05$). [a,b]Means with unlike superscripts within each sampling time are different ($P = 0.02$).

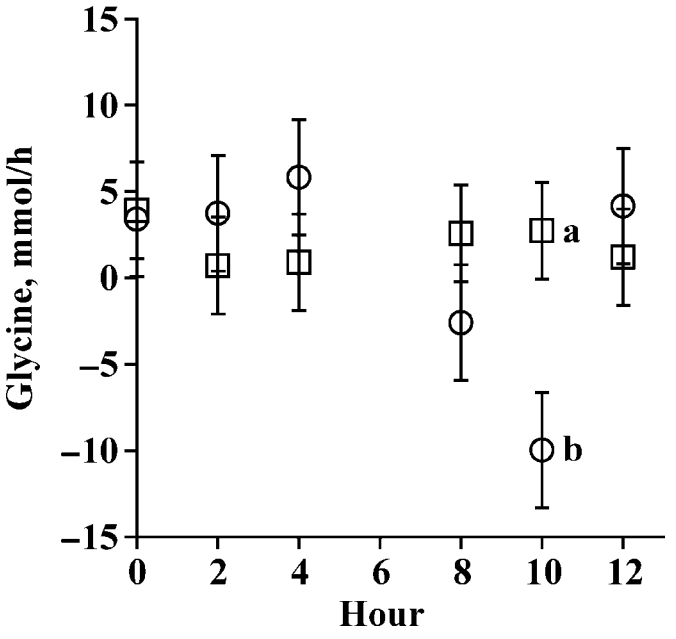

Figure 4. Portal-drained viscera flux [least squares means ± SEM (bars)] of Gly in nulliparous yearling Rambouillet crossbred ewes that were fed either low (○; 2.28 g/d) or high (□; 3.91 g/d) metabolizable Met. Sampling time × metabolizable Met interaction was significant ($P = 0.02$). [a,b]Means with unlike superscripts within each sampling time are different ($P < 0.01$).

cifically, in inoculated ewes, vena caval-blood neutrophil, lymphocyte, monocyte, and eosinophil numbers were significantly altered (Lewis, 2003). Based on these immediate immune responses, repeatability, and ease of application, this method seemed appropriate for achieving the objectives of the present experiment. As a result, acute sepsis was successfully induced in inoculated ewes. Indicators of sepsis, in addition to elevated body temperatures, were alterations in AA metabolism (discussed below) and ability to culture bacteria from the inoculated uteri (postmortem).

Supporting Sepsis-Induced Anabolism

To support immune-system activation, anabolic and catabolic processes occur simultaneously. For example, AA from muscle-protein catabolism are used for immune-responsive synthesis of products such as acute-phase proteins and glutathione. In the current study, such sepsis-induced effects are evident based on 2 major observations for inoculated ewes: 1) sustained hepatic

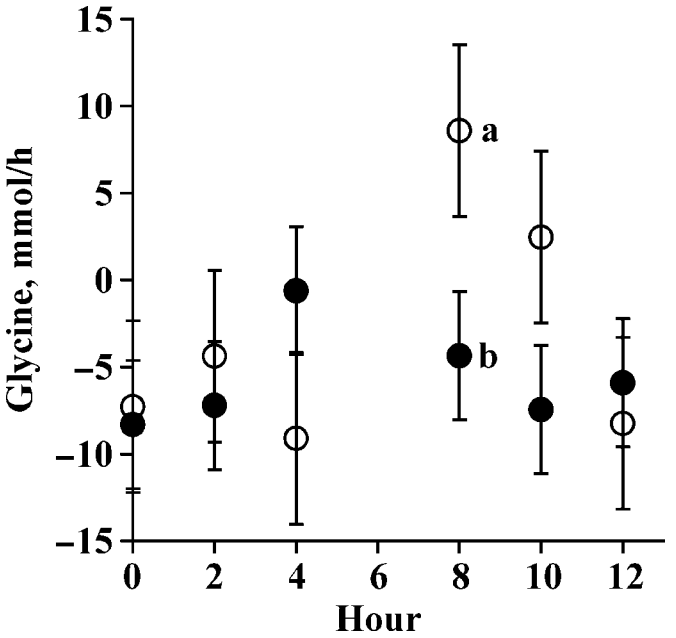

Figure 5. Hepatic flux [least squares means ± SEM (bars)] of Gly in nulliparous yearling Rambouillet crossbred ewes that received an intrauterine sterile-saline infusion (○) or bacterial inoculation (●; 9.69×10^{11} cfu of *Escherichia coli* and 2.76×10^{12} cfu of *Arcanobacterium pyogenes*). Sampling time × inoculation interaction was significant ($P = 0.05$). [a,b]Means with unlike superscripts within each sampling time are different ($P < 0.01$).

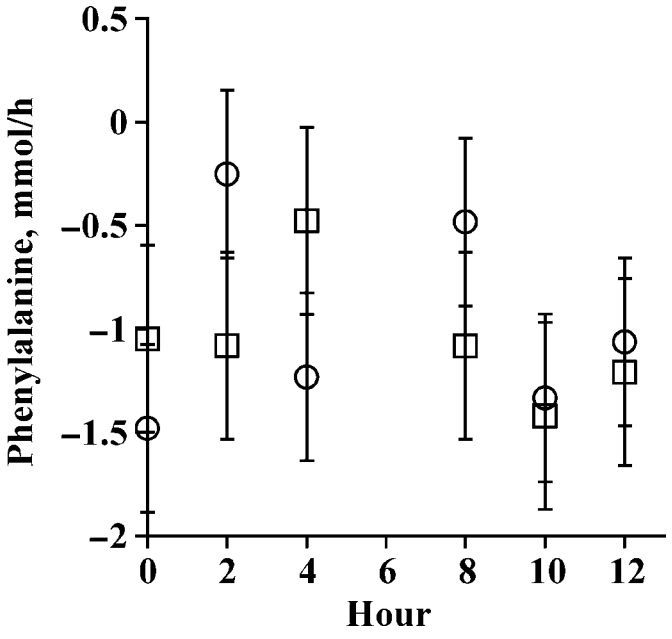

Figure 6. Hepatic flux [least squares means ± SEM (bars)] of Phe in nulliparous yearling Rambouillet crossbred ewes that were fed either low (○; 2.28 g/d) or high (□; 3.91 g/d) metabolizable Met. Sampling time × metabolizable Met interaction was significant (P = 0.04).

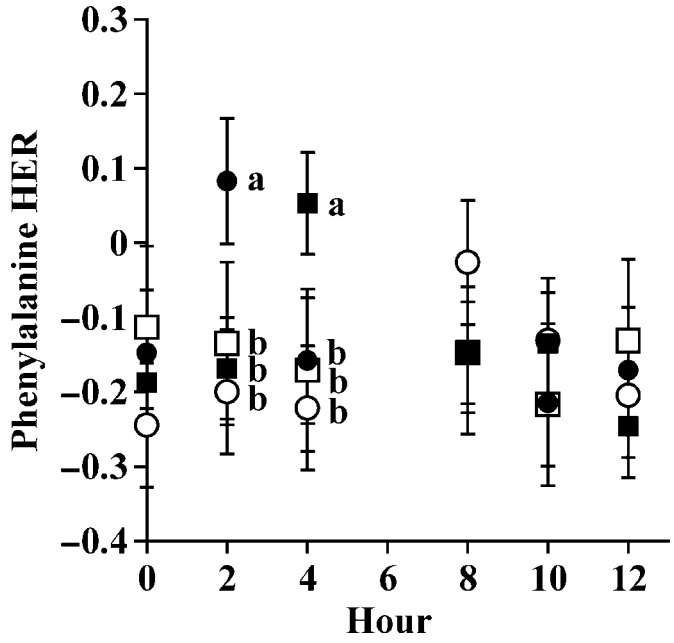

Figure 7. Hepatic extraction ratio (HER) [least squares means ± SEM (bars)] of Phe in nulliparous yearling Rambouillet crossbred ewes that were: 1) fed low dietary metabolizable Met (2.28 g/d) and received an intrauterine saline infusion (○), 2) fed high metabolizable Met (3.91 g/d) and received an intrauterine saline infusion (□), 3) fed low dietary metabolizable Met (2.28 g/d) and received an intrauterine bacterial inoculation (●; 9.69 × 10^{11} cfu of *Escherichia coli* and 2.76 × 10^{12} cfu of *Arcanobacterium pyogenes*), or 4) fed high metabolizable Met (3.91 g/d) and received an intrauterine bacterial inoculation (■; 9.69 × 10^{11} cfu of *E. coli* and 2.76 × 10^{12} cfu of *A. pyogenes*). Sampling time × metabolizable Met × inoculation interaction was significant (P = 0.03). [a,b]Means with unlike superscripts within each sampling time are different (P < 0.05).

uptake of Gly over time and 2) greater proportional hepatic release of Phe.

In noninoculated ewes, hepatic tissue transitioned from net uptake (h 0 and 2) to net release of Gly (h 8 and 10). However, intrauterine bacterial inoculation seemed to inhibit net release of Gly, resulting in a sustained hepatic uptake throughout the 12-h sampling period. This response, coupled with decreased artery plasma Met and Ser and hepatic-vein plasma Glu, indicates increased glutathione synthesis. In rats receiving i.v. injection of *E. coli,* glutathione production increased 3.4-fold with the greatest response occurring in hepatic tissue (Malmezat et al., 2000a). This increased rate of glutathione synthesis accounted for 38% of Cys used for protein synthesis. In the current study, intrauterine bacterial inoculation did not influence Cys flux across splanchnic tissues or concentration in plasma. Thus, it seems that Cys was not limiting, regardless of the level of MM, in inoculated ewes. Compared with noninoculated ewes, the lower Met concentrations in artery and portal-vein plasma may have been, in part, because of increased transsulfuration of Met. Partial demand of Cys used for glutathione synthesis in septic rats (discussed above and by Malmezat et al., 2000b) was from increased Met transsulfuration. Although inoculation treatment did not alter Glu flux, it did decrease the concentration in hepatic-vein plasma when compared with noninoculated ewes. Taken together, we suggest that Gly was used to support sepsis-induced glutathione synthesis.

Regardless of level of MM, hepatic tissue in noninoculated ewes consistently and proportionally (based on the HER) released less Phe than was received from PDV and artery plasma. Conversely, intrauterine inoculation resulted in proportionally more Phe released from the hepatic tissue 14 (low MM) and 16 (high MM) h after inoculation. Increased plasma Phe during sepsis has been documented in humans (for review see Jeevanandam, 1995); it is a result of peripheral-protein catabolism, mainly skeletal muscle protein. In artery and portal-vein plasma of inoculated ewes, the Phe:Tyr ratio was 1.2 compared with 0.9 for noninoculated ewes (inoculation treatment effect, P = 0.08 and 0.01, respectively), which indicates that intrauterine bacterial inoculation resulted in movement of Phe from protein to plasma via catabolism, most likely, of skeletal muscle protein (Wannemacher et al., 1976). Many of the immune-response (e.g., acute phase) proteins synthesized in the hepatic tissue contain substantially more Phe, Tyr, and Trp than does skeletal muscle protein (Reeds et al., 1994). In rats, presence of sepsis does not influ-

ence renal clearance rate or hepatic oxidation of Phe, but impedes its incorporation into skeletal muscle (Wannemacher et al., 1976). In the current study, we suggest that sepsis-induced peripheral release of Phe (i.e., increased arterial Phe) was greater than the hepatic demand of Phe for sepsis-induced protein synthesis (e.g., acute phase proteins).

Supply of MM

The supply of additional MM from the commercial rumen-protected feed product, Mepron M85, did not alter net PDV flux of Met. This suggests that 1) the supply of MM was less than that estimated (Table 1) from product specifications, or 2) loss of experimental units due to catheter-patency complications (Table 2) limited our ability to detect differences in Met flux. An examination of the bioavailability of several different ruminally protected Met sources revealed that Mepron M85 was least effective at raising plasma Met concentrations (Südekum et al., 2004), which confirmed earlier findings (Blum et al., 1999). Regardless, altered fluxes of blood metabolites and altered arterial concentrations of other AA for the high-MM vs. low-MM treatment indicated that the rumen-protected product used in this study supplied some absorbable Met.

Need for MM

In the context of this study, it is important to decipher, first, if additional MM was needed, regardless of sepsis, in low-MM ewes. Two events that occurred indicate that Met may have been limiting in low-MM ewes: 1) sustained net hepatic uptake of Phe in high-MM ewes and 2) a net release of Asp accompanied with greater hepatic-vein plasma glucose in high-MM ewes. High-MM resulted in greater portal-vein plasma Phe. Additional MM may have been needed in low-MM ewes, which would have ensured use of Phe for protein synthesis in hepatic tissue and, possibly, elsewhere. If Met was needed, breakdown of "excess" AA would be expected to occur resulting in increased ureagenesis. In high-MM ewes, compared with low-MM, hepatic-vein glucose concentration was greater, and there was a net hepatic release of Asp. Thus, ureagenesis may have been greater in low-MM ewes, although differences in urea flux were not observed. Taken together, it seems that low-MM ewes needed additional dietary Met.

Still unanswered is whether the presence of an acute infection in inoculated ewes prompted a need for additional MM and whether Met was the most limiting AA during sepsis. In low-MM ewes, arterial plasma Trp remained unchanged regardless of inoculation treatment. But, when MM was increased in the diet, artery plasma Trp was less in noninoculated ewes than inoculated. As with Phe, Trp is required for synthesis of acute-phase proteins and its removal from the plasma is mainly either through incorporation into protein or breakdown in hepatic tissue (Reeds et al., 1994; Le Floc'h et al., 2004). Because high MM did not affect rises in artery plasma Trp in inoculated ewes, Met availability did not seem to be the limiting factor during sepsis. Human skeletal-muscle protein is rich in Met (Reeds et al., 1994). Assuming the same for ovine muscle, the fact that, gram for gram, more Met-rich muscle protein must be mobilized to ensure availability of aromatic AA for acute phase protein synthesis, Met would not necessarily be limiting in ewes experiencing acute sepsis. Therefore, when MM is sufficient for nulliparous yearling ewes, additional Met may not provide any benefit to a ewe experiencing acute sepsis.

Concluding Remarks

Although somewhat described in nonruminant organisms, the effects of acute sepsis on splanchnic AA metabolism in ruminants are largely unknown. The lack of comparative data in the literature presents challenges when attempting to link observed effects to physiological processes. Nevertheless, using an established intrauterine bacterial inoculation method, we induced acute sepsis in nulliparous yearling ewes, while simultaneously measuring AA metabolism in splanchnic tissues. For some measurements, the number of treatment experimental units available for statistical analyses were small; thus, some effects of inoculation may have occurred undetected. However, inoculation resulted in increased plasma concentrations of Phe, increased hepatic removal and decreased plasma concentrations of AA important for glutathione synthesis, and decreased plasma concentrations of some gluconeogenic and acetogenic AA. We also investigated the effects of feeding diets low or high in MM on splanchnic AA metabolism in septic ewes. Supplemental Met above what is physiologically needed would not necessarily result in a benefit to septic ewes.

LITERATURE CITED

AOAC. 1990. Official Methods of Analysis. 15th ed. Assoc. Off. Anal. Chem., Arlington, VA.

Arias-Díaz, J., E. Vara, C. Garcia, N. Villa, J. M. Rodriguez, P. Ortiz, and J. L. Balibrea. 1996. S-adenosylmethionine protects hepatocytes against the effects of cytokines. J. Surg. Res. 62:79–84.

Blum, J. W., R. M. Bruckmaier, and F. Jans. 1999. Rumen-protected methionine fed to dairy cows: Bioavailability and effects on plasma amino acid pattern and plasma metabolite and insulin concentrations. J. Dairy Sci. 82:1991–1998.

Chen, L., W. Allen, and J. R. Strickland. 2002. Determination of free amino acids in serum by capillary gas chromatography. Proc. West. Sec. Am. Soc. Anim. Sci. 53:556–560.

Greenwood, R. H., and E. C. Titgemeyer. 2000. Limiting amino acids for growing Holstein steers limit-fed soybean hull-based diets. J. Anim. Sci. 78:1997–2004.

Hofford, J. M., L. Milakofsky, S. Pell, and W. Vogel. 1996. A profile of amino acid and catecholamine levels during endotoxin-induced acute lung injury in sheep: Searching for potential markers of the acute respiratory distress syndrome. J. Lab. Clin. Med. 128:545–551.

Huntington, G. B. 1982. Portal blood flow and net absorption of ammonia-nitrogen, urea-nitrogen, and glucose in nonlactating Holstein cows. J. Dairy Sci. 65:1155–1162.

Jeevanandam, M. 1995. Trauma and sepsis. Pages 245–255 in Amino Acid Metabolism and Therapy in Health and Nutritional Disease. L. A. Cynober, ed. CRC Press Inc., Boca Raton, FL.

Jung, D., H. Biggs, J. Erikson, and P. U. Ledyard. 1975. New colorimetric reaction for end-point, continuous-flow, and kinetic measurement of urea. Clin. Chem. 21:1136–1140.

Le Floc'h, N., D. Melchior, and C. Obled. 2004. Modifications or protein and amino acid metabolism during inflammation and immune system activation. Livest. Prod. Sci. 87:37–45.

Lewis, G. S. 2003. Role of ovarian progesterone and potential role of prostaglandin $F_{2\alpha}$ and prostaglandin E_2 in modulating the uterine response to infectious bacteria in postpartum ewes. J. Anim. Sci. 81:285–293.

Lewis, G. S., and M. C. Wulster-Radcliffe. 2006. Prostaglandin F2α up regulates uterine immune defenses in the presence of the immunosuppressive steroid progesterone. Am. J. Reprod. Immunol. 56:102–111.

Malmezat, T., D. Breuillé, C. Pouyet, C. Buffière, P. Denis, P. P. Mirand, and C. Obled. 2000a. Methionine transsulfuration is increased during sepsis in rats. Am. J. Physiol. Endocrinol. Metab. 279:E1391–E1397.

Malmezat, T., D. Breuillé, C. Pouyet, P. Capitan, P. P. Mirand, and C. Obled. 2000b. Glutathione turnover is increased during the acute phase of sepsis in rats. J. Nutr. 130:1239–1246.

Mortenson, M. C., G. E. Schuman, L. J. Ingram, V. Nayigihugu, and B. W. Hess. 2005. Forage production and quality of a mixed-grass rangeland interseeded with *Medicago sativa* ssp. falcata. Range Ecol. Manage. 58:505–513.

Neeley, W. E., and J. Phillipson. 1988. Automated enzymatic method for determining ammonia in plasma, with 14-day reagent stability. Clin. Chem. 34:1868–1869.

Noll, F. 1974. Page 1465 in Methods of Enzymatic Analysis, 2nd ed. Academic Press, New York, NY.

NRC. 1996. Nutrient Requirements of Beef Cattle. 7th rev. ed. Natl. Acad. Press, Washington, DC.

Ramadan, A. A., G. L. Johnson, and G. S. Lewis. 1997. Regulation of uterine immune function during the estrous cycle and in response to infectious bacteria in sheep. J. Anim. Sci. 75:1621–1632.

Reeds, P. J., C. R. Fjeld, and F. Jahoor. 1994. Do the differences between the amino acid compositions of acute-phase and muscle proteins have a bearing on nitrogen loss in traumatic stress? J. Nutr. 124:906–910.

Reeds, P. J., and F. Jahoor. 2001. The amino acid requirements of disease. Clin. Nutr. (Suppl. 1):15–22.

Seals, R. C., M. C. Wulster-Radcliffe, and G. S. Lewis. 2003. Uterine response to infectious bacteria in estrous cyclic ewes. Am. J. Reprod. Immunol. 49:269–278.

Seals, R. C., M. C. Wulster-Radcliffe, and G. S. Lewis. 2002. Modulation of the uterine response to infectious bacteria in postpartum ewes. Am. J. Reprod. Immunol. 47:57–63.

Sheldon, I. M., G. S. Lewis, S. Leblanc, and R. O. Gilbert. 2006. Defining postpartum uterine disease in cattle. Theriogenology 65:1516–1530.

Song, Z., S. Barve, T. Chen, W. Nelson, S. Uriarte, D. Hill, and C. McClain. 2002. S-adenosylmethionine (AdoMet) modulates endotoxin stimulated interleukin-10 production in monocytes. Am. J. Physiol. Gastrointest. Liver Physiol. 284:G949–G955.

Song, Z., S. Uriarte, R. Sahoo, T. Chen, S. Barve, D. Hill, and C. McClain. 2005. S-adenosylmethionine (SAMe) modulates interleukin-10 and interleukin-6, but not TNF, production via the adenosine (A2) receptor. Biochim. Biophys. Acta 1743:205–213.

Storm, E., and E. R. Ørskov. 1983. The nutritive value of rumen microorganisms in ruminants. 1. Large-scale isolation and chemical composition of rumen micro-organisms. Br. J. Nutr. 50:463–470.

Storm, E., and E. R. Ørskov. 1984. The nutritive value of rumen micro-organisms in ruminants. 4. The limiting amino acids of microbial protein in growing sheep determined by a new approach. Br. J. Nutr. 52:613–620.

Südekum, K. H., S. Wolffram, P. Ader, and J. C. Robert. 2004. Bioavailability of three ruminally protected methionine sources in cattle. Anim. Feed Sci. Technol. 113:17–25.

Taylor, J. B. 2000. Disposition of swainsonine and its effects on nutrient metabolism in ruminants. PhD Diss. New Mexico State Univ., Las Cruces.

Trinder, P. 1969. Determination of blood glucose using an oxidase-peroxidase system with a non-carcinogenic chromogen. J. Clin. Pathol. 22:158–161.

Wannemacher, R. W. 1977. Key role of various individual amino acids in host response to infection. Am. J. Clin. Nutr. 30:1269–1280.

Wannemacher, R. W., A. S. Klainer, R. E. Dinterman, and W. R. Beisel. 1976. The significance and mechanism of and increased serum phenylalanine-tyrosine ratio during infection. Am. J. Clin. Nutr. 29:997–1006.